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Differential inhibition using contextual stimuli

J.H.R. Maes *, J.M.H. Vossen

Department of Comparative and Physiological Psychology, University of Nijmegen, P.O. Box 9104, 6500 HE Nijmegen, Netherlands

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Abstract

The present experiments examined whether external contextual stimuli can acquire inhibitory properties through a simple differential context-reinforcement procedure. Rats first received discrimination training sessions in which an electric footshock was consistently delivered in one context, but not in a second, different context. In Experiments 1A and 1B, summation and retardation tests were subsequently performed using the non-reinforced context. Each of these tests failed to reveal contextual conditioned inhibition. In Experiment 2, a summation and retardation test were performed using a contextual stimulus that was unique to the non-reinforced context. Weak but significant contextual inhibition was found. It was suggested that contextual stimuli that are unique to the non-reinforced context did acquire inhibitory strength in each experiment, but that in Experiments 1A and 1B, expression of this inhibition during testing had been masked by the concurrent presence of excitation from elements that the non-reinforced context had in common with the reinforced context.

Keywords: Common elements; Contextual stimuli; Differential inhibition; Freezing; Rats; Unique elements

1. Introduction

Numerous studies on associative learning demonstrate that contextual stimuli can become directly associated with an unconditioned stimulus (US). For instance, presenting an electric footshock to a rat in a particular box results in the rat's acquisition of a fear response to that box. This response reflects a simple association between box and shock (e.g., Fanselow and Tighe, 1988; Maes and Vossen, 1992). Contextual stimuli can even acquire direct excitatory associative strength in a standard classical conditioning experiment in which a discrete stimulus provides a more predictive signal for the occurrence of a US than does the context (e.g., Maes and Vossen, 1993a; Williams et al., 1992).

* Corresponding author. Tel: +31 (24) 3612544; Fax: +31 (24) 3616066; E-mail: maes@nici.kun.nl

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In the literature on animal learning, either explicitly or implicitly, reference has been made to the possibility that external contextual stimuli can also acquire a direct inhibitory associative strength (see, e.g., formal models of associative learning like that of Mackintosh, 1975; Pearce, 1987; Pearce and Hall, 1980; Rescorla and Wagner, 1972). To the authors' knowledge, however, there are no studies that clearly demonstrate such contextual inhibition (see Cunningham, 1979, for one demonstration of inhibition conditioned to an 'internal' context provided by alcohol). For compelling evidence of inhibition it is generally accepted that the putative inhibitor must pass both retardation and summation tests for conditioned inhibition (Rescorla, 1969). Regarding contextual inhibition, this implies that contextual stimuli must have the potential to attenuate conditioned responding that is elicited by any excitatory stimulus that has been trained with the same US (summation test), and the acquisition of excitatory responding to the contextual stimuli through pairings with the US, must be impaired (retardation test).

There are at least two conditioning procedures that, theoretically, should result in contextual inhibition. The first procedure is based on the Pavlovian A + /AB − conditioned inhibition procedure in which stimulus A is reinforced by a US when presented in isolation, and non-reinforced when accompanied by stimulus B. In this case, B will be transformed into an inhibitor. A parallel procedure using contextual stimuli would be an AX + /AY − discrimination procedure in which a discrete stimulus A is followed by a US in context X, and not followed by a US in a distinctively different context Y. Context Y potentially could acquire inhibitory power because, as in the Pavlovian A + /AB − procedure, the intended inhibitor (Y) is non-reinforced in the presence of an excitor (A). However, using summation and retardation tests, there is no empirical proof that AX + /AY − training results in Y becoming a conditioned inhibitor. Instead, Y seems to acquire the potential to 'set the occasion' for non-reinforcement of A by signalling or retrieving the A-no-US relationship; it does not signal or retrieve the 'no-US event' directly, as is the case for conditioned inhibitors (e.g., Bouton and Swartzentruber, 1986; see also Maes and Vossen, 1993b).

A second procedure that, in principle, could yield contextual inhibition is based on differential conditioning. Differential reinforcement of two stimuli A and B, which can be symbolized as A + /B − , has frequently been shown to result in the non-reinforced stimulus acting as a conditioned inhibitor, especially in the case that the two stimuli are of the same modality (see LoLordo and Fairless, 1985, for a review). Consequently, it might be that differential reinforcement of two contexts X and Y that differ on one dimension only, such as, for instance, the presence of an auditory stimulus, also culminates in context Y acquiring conditioned inhibitory strength. Differential reinforcement of contexts has previously been applied in studies that were not explicitly designed to examine contextual inhibition (e.g., Good and Honey, 1991), and separate tests for conditioned inhibition were not performed.

The purpose of the present experiments was to assess whether or not X + /Y − training using contexts results in Y acquiring an inhibitory potential, and, more specifically, to evaluate under which circumstances such potential may be uncovered. According to the formal models of associative learning mentioned above, a condition that necessarily must be met in order for a stimulus to acquire inhibitory associative strength is that the intended conditioned inhibitor is non-reinforced in the presence of an excitatory stimulus. It is assumed that in the standard A + /B − differential conditioned inhibition procedure using discrete stimuli, the common context in which both reinforced and non-reinforced trials occur constitutes this necessary excitatory element (LoLordo and Fairless, 1985; Miller et al., 1991).

It may be argued that in a X + /Y − contextual training procedure the stimuli that context Y has in common with context X constitute the necessary excitatory stimuli in the presence of which the elements that are unique to context Y are non-reinforced. Assuming that this circumstance is sufficient for Y's unique stimuli to acquire inhibitory strength, it is then impossible to demonstrate this inhibition if all elements of context Y are simultaneously tested. The problem is that expression of the inhibition acquired by the unique elements of Y is masked by the excitation from common elements.

In Experiments 1A and 1B, we tried to establish that X + /Y − differential contextual reinforcement indeed does not reveal any inhibition by Y if all stimuli that constitute Y are simultaneously tested. In Experiment 2, we adopted a testing procedure in which an element that was unique to context Y was separately subjected to tests for conditioned inhibition. It was expected that this procedure would uncover contextual inhibition.

2. Experiments 1A and 1B. Summation and retardation tests using the non-reinforced context

In both Experiments 1A and 1B, rats were first subjected to a X + /Y − contextual discrimination training in which a footshock occurred after placement in context X, but not after placement in context Y. The response observed was freezing, a species-specific defense reaction (e.g., Fanselow and Tighe, 1988). After establishment of differential freezing in the two contexts, the rats received a summation test in Experiment 1A, in which an excitatory tone was presented in X, Y, and the home cage (HC). If Y has acquired inhibitory properties, conditioned freezing to the tone should be less in context Y than in the excitatory context X, or the neutral HC. In Experiment 1B, a retardation test was performed following discrimination training. Shocks were presented in context Y and in a neutral context Z. Potential inhibition acquired by Y should be revealed by a slower acquisition of conditioned freezing in context Y than in context Z.

2.1. Materials and methods

2.1.1. Subjects and apparatus

Twenty-seven experimentally naive female Wistar rats served as the subjects. Their mean body weight ranged from 221 to 416 g. The animals were housed individually in Makrolon cages in which they had free access to food and water. The rats were maintained on a 12-h dark/12-h light schedule (lights off: 8.00 a.m.), and all experimental manipulations were performed during the dark period.

Three contexts were used. One context consisted of a box measuring 24.5 × 25 × 24 cm. The front and back wall, as well as the top were constructed of clear Plexiglas, and the two side walls were made of aluminium. The floor of the box was composed of 3-mm stainless steel rods spaced 1.3 cm apart. The US used was a 0.5-mA, 0.5-s scrambled electric shock presented through the rods. A speaker was located on the left side of one side wall. This speaker could be used for presenting a 30-s continuous tone (75 dB(A), 7 kHz). The box was inserted in a sound-attenuating chest which had a clear Plexiglas front window that enabled the observation of an animal inside. The box was illuminated by a light bulb mounted to the ceiling of the chest. The light intensity measured inside the box was 118 cd/m^2 (Minolta luminance meter LS-100 directed at light source). A distinctive olfactory cue was provided by a piece of wadding scented with cleaning alcohol placed in the environmental chest.

The second box was identical to the box described above, except for the following. The box was placed on a table in a room that was illuminated by two red fluorescent tubes (light intensity inside the box: < 1 cd/m^2). The back wall was gray plastic with vertical black stripes. A speaker was located on the right side of one side wall and no specific olfactory cue was introduced in this box.

The walls of the third box were made of Plexiglas and formed a diamond-like shape. Two white Plexiglas parts (20×30 cm) were fixed to each other so as to form an angle of 70°. To each end of these parts, another white plastic piece (10×30 cm) was attached forming an angle of 105° with the other part. A piece of clear plastic (11×30 cm) was used to complete the wall. The floor of the box consisted of identical rods as described for the other two boxes. On the clear plastic lid that covered the box, a speaker was attached that could be used for presenting auditory stimuli. The box was located in a room that was illuminated by four white fluorescent tubes (light intensity inside the box: 3 cd/m^2). No distinctive odour was introduced.

To enable assessment of conditioned responding to the tone in the HC in Experiment 1A, a speaker was placed on top of the wire lid of the cage. The speaker was wired to the same tone generator as was used for presenting the tone in each of the three contexts described above. Presentations of the US and of the tone were controlled by a Macintosh/SE computer.

2.1.2. Procedure

2.1.2.1. Experiment 1A

Differential conditioning to contexts. Prior to differential context conditioning, each animal ($n = 9$) was exposed to context X and context Y. The actual type of box that provided these contexts was counterbalanced so that for five animals X was the first box described in Section 2.1.1, and Y the second, whereas for the remaining four rats, X was the second, and Y was the first box. Exposure to each context lasted 6 min. During the following phase, all rats received two types of session. One session was performed in context X and lasted 6 min. The animal was placed in X (start of the session) and after a mean interval of 163 s, one shock US was presented. The other session was conducted in context Y and merely consisted of placing the rat in the appropriate box for 6 min without the footshock US being delivered. The order of the sessions in X and Y was randomized with the restriction that no more than two consecutive training sessions were performed in the same context. Two training sessions were performed each day (inter-session interval about 3 h). A total of 10 reinforced, and 10 non-reinforced sessions was given.

Tone conditioning and context reconditioning. Following discrimination training, each rat was individually placed in context Z, which consisted of the third context described above, and received four pairings of the 30-s tone and the footshock US (inter-stimulus interval: 2.5 min). The shock was presented during the last 0.5 s of each tone presentation. Approx. 10 s after termination of the last tone, the animal was removed from Z and returned to the HC. On the next day, the animals received one standard unsignalled shock-presentation session in X (shock after 150 s) and one non-reinforced context Y exposure session.

Test for responding to the tone in X, Y, and HC. The test phase consisted of two non-reinforced presentations of the tone in each of the contexts X and Y, and in the HC. The first 30-s tone in each context was presented 2.5 min after the start of the session. The second tone was presented 1.5 min after termination of the first, and each session ended 1 min thereafter. The sessions were performed on 3 consecutive days. The order of test contexts was counterbalanced as completely as possible. The session in the HC consisted of bringing the rat to the experimental room (while it remained in the HC), placing the speaker on the lid of the HC, and presenting the two non-reinforced tones. The experimental room consisted of the same room as used for placing contexts X, Y, and Z. During HC sessions, this room was illuminated by two white fluorescent tubes (5 cd/m^2) and the HC was placed on a table situated in a part of the room that differed from that used when employing each of the other contexts.

2.1.2.2. Experiment 1B

Exposure to context Z. For two sessions, which were separated by approx. 24 h, each of the 18 animals included in Experiment 1B was placed in context Z for 0.5 h.

Differential conditioning to contexts. The animals received discrimination training sessions identical to those described in Experiment 1A, except that the interval between sessions was 24 h. The physical boxes that provided X, Y, and Z were counterbalanced between the three boxes.

Exposure to Z. To assess the associative status of context Z, which was to be used as a comparison context in the retardation test, one 6-min context Z exposure session was conducted (no events planned) after discrimination training. In principle, this single non-reinforced session could have resulted in Z (or better: Z's unique elements) acquiring some inhibitory potentials. However, it was assumed that this inhibition would be much weaker than that potentially acquired by context Y, which had been part of the discrimination procedure more explicitly.

Retardation tests. Two groups of rats were formed ($n = 9$ for each group), matched for the freezing difference score based on the last two discrimination training sessions. The difference score was of the form: percentage of observations scored as freezing during the first minute of the last session in X minus percentage of observations scored as freezing during the first minute of the last session in Y. Matching was also performed with respect to physical identity of contexts Y and Z. On each of 5 days, one group, group Y +, was placed in context Y where the footshock US was presented after a mean of 181 s. The animals stayed in context Y for a total of 6 min per session. The other group of rats, group Z +, received the same treatment as group Y +, except that the sessions took place in context Z.

Dependent measure and statistical analyses. Aided by a light stimulus that was not visible to the rats, behaviour was scored as freezing or not freezing every fifth second (time sampling; the observation window was 1 s). The behaviour was defined as freezing if the animal was completely motionless. The only two movements that were allowed to occur in this behavioural category were (1) movements caused by respiration, and (2) a slow, rhythmical horizontal movement of the head that typically occurs in emotionally excited albino rats (see, e.g., Kolpakov et al., 1977). All other behaviour was scored as not freezing. During the critical tests in the different contexts, the observer was unaware of whether the test context was context X or context Y (Experiment 1A), or of the rats' group assignments (Experiment 1B).

Freezing scores were subjected to analyses of variance (ANOVAs). Where appropriate, interactions between main effects were analysed using simple main-effect analyses. The error term and degrees of freedom that were used in these latter tests were derived from the overall analysis (Winer, 1971). An alpha level of 0.05 was selected for all tests of statistical significance.

2.2. Results

2.2.1. Differential conditioning to contexts (Experiments 1A and 1B)

The dependent measure used as an index of context fear during discrimination training was the percentage of observations scored as freezing during the first minute of each training session. During this period, the US had not yet been presented in X.

In both Experiments 1A and 1B, discrimination training proceeded smoothly. During the first session in X and Y of Experiment 1A, the rats froze on 9.3% and 5.6% of the first-minute observations in X and Y, respectively. The difference was not significant. On the final session of discrimination training, the rats froze on 70.4% of the observations in X, and on only 15.7% of the observations in Y. Now the difference was highly significant ($F(1, 8) = 28.07$). In Experiment 1B, an identical pattern of results was observed. The freezing percentages were 1.9 and 7.9 during the first training sessions in X and Y, respectively, and the difference was insignificant. During the final sessions, the corresponding percentages were 69.0 and 13.0. This difference was highly reliable ($F(1, 17) = 55.84$). These results indicate that in both experiments discrimination training was successful.

2.2.2. Experiment 1A

2.2.2.1. Tone conditioning and context reconditioning. The rats froze on 5.6% of the observations during the first minute of the tone conditioning session. The freezing percentages observed during each of the four tone presentations were 24.1, 51.9, 40.7, and 53.7. The increase in freezing to the tone was significant ($F(3, 24) = 6.26$), indicating successful tone conditioning.

During the retraining discrimination sessions (session 11 in X and Y), the subjects froze on 64.8% of the observations made during the first minute in context X, and on 13.0% during the first minute in context Y. The difference was significant ($F(1, 8) = 22.56$). Thus, discrimination performance had not been affected by the previous tone conditioning session.

2.2.2.2. Tests for responding to the tone. The associative status of each of the different test contexts prior to presentations of the tone was assessed by examining freezing during the very first minute after placement. The left panel of Fig. 1 shows the corresponding data, as well as the CS freezing levels in each of the test contexts.

The data shown are pooled over the factors Identity of context Y and Test Order because each of these factors was not significant and did not reliably interact with any other factor.

The figure indicates that context X was the most excitatory, followed by context Y, which had a low excitatory potential, and, finally, the HC in which freezing was virtually absent. More importantly, CS freezing was comparable in the different test contexts. A Period (first minute or CS period) $\times$ Context ANOVA performed on the data depicted in the right panel of Fig. 1 revealed a significant effect of Period ($F(1, 8) = 20.73$), Context ($F(2, 16) = 15.49$), and Period $\times$ Context interaction ($F(2, 16) = 13.64$). Simple main effect analyses revealed that the rats froze differentially in the test contexts during the first minute of the test sessions ($F(2, 32) = 28.78$), but not during the CS presentations of these sessions ($F(2, 32) = 1.39$). Newman–Keuls tests revealed that the rats during the first minute froze more in context X than in context Y or in the HC ($P\text{s} < 0.01$). The difference between freezing in context Y and freezing in the HC was not significant ($P > 0.05$).

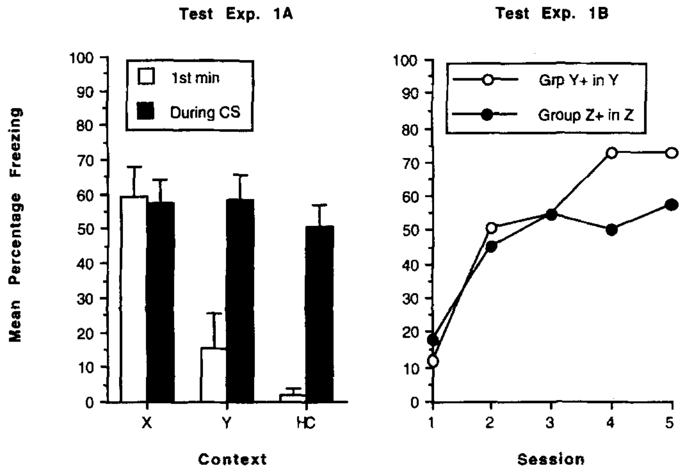

Fig. 1. Left: mean percentage (+S.E.M.) of observations scored as freezing in Experiment 1A during the first minute in each of the CS test contexts X, Y, and HC (home cage), and during the tone-CS presentations in these contexts. X was the context previously used in a differential context-conditioning procedure as the reinforced context and Y had previously been the non-reinforced context. Right: mean percentage of observations scored as freezing during the first minute of the reinforced test sessions in Y (group Y+), and Z (group Z+) of Experiment 1B. Context Y was the non-reinforced context from a differential context-conditioning procedure and context Z was a putative neutral context.

Collectively, the analyses confirm that the level of tone freezing was not significantly affected by differences in the associative status of the test context.

2.2.3. Experiment 1B

2.2.3.1. Test Z and retardation tests. Freezing during the first minute of the 6-min context Z exposure session was compared with first-minute freezing of the last session in each of the contexts X and Y. In group Y+, the mean percentages in X, Y, and Z were 73.2, 16.7, and 25.0, respectively. In group Z+, the corresponding mean percentages were 64.8, 9.3, and 38.0. A Group × Context ANOVA only revealed a significant main effect of Context ($F(2, 32) = 28.67$; other Fs < 1.28). Subsequent Newman–Keuls tests found that the rats froze more in context X than in each of the contexts Y and Z (Ps < 0.01). Furthermore, the animals froze more in context Z than in context Y (P < 05).

The right portion of Fig. 1 depicts the results of the retardation test. A Group × Session repeated measures ANOVA uncovered a significant effect of Session ($F(4, 64) = 19.01$). The effect of Group, and the interaction between Group and Session were not significant ($F(1, 16) < 1$ for Group, and $F(4, 64) = 1.59$ for the interaction), indicating no difference in rate of conditioning between groups.

2.3. Discussion

After employing an X+/Y− differential contextual reinforcement procedure, context Y did neither pass a summation nor a retardation test for conditioned inhibition.

Previous studies show that, using contexts that by means of some direct measurement of associative strength (e.g., context-preference tests) prove to possess excitatory strength, there is no summation of the context's associative potential and that of a consistently reinforced discrete stimulus (e.g., Bouton, 1993). Thus, at least with shock as a US, responding to an excitatory stimulus in an excitatory context does not differ from responding to that stimulus in an associatively neutral context. The results of the tone tests of Experiment 1A are consistent with these data. Animals during the last tone presentation of the conditioning session in context Z responded on 53.7% of the observations, which is a level of responding that does not differ much from that observed in each of the test contexts.

In the light of the foregoing, it may be argued that the summation test used in Experiment 1A is not the most appropriate test for Y's inhibitory potential. If contexts do not readily summate their associative strength with that of standard discrete Pavlovian conditioned stimuli (CS), conditioned inhibition acquired by Y may not be detected by a summation test, even though other tests would have uncovered such inhibition. This problem was avoided in Experiment 1B, in which a test for conditioned inhibition was used that does not rely on summation of associative strengths of contextual and discrete stimuli. However, this experiment too failed to uncover contextual inhibition.

A final point of discussion concerns the use of the HC as a 'neutral' test context. It might be argued that the HC is at least as 'safe' a box as is the non-reinforced context Y. Consequently, the HC might not constitute a sensitive comparison environment for detecting inhibition in Experiment 1A. However, the critical point is that the HC is neutral with respect to the shock US employed. That is, it is reasonable to assume that, as the training phase was in effect using contexts X and Y, in the HC there were no, or very few, excitatory, common shock-associated elements that would provide a basis for the development of a conditioned inhibitory property by the HC. Only context Y, then, could, in principle, acquire an 'active', conditioned response-inhibiting property.

3. Experiment 2. Retardation and summation tests using a unique contextual stimulus of the non-reinforced context

An evaluation of the null result of Experiments 1A and 1B may be based on the analysis outlined in Section 1. Accordingly, elements that were unique to context Y did acquire an inhibitory potential, but this inhibition could not be detected in the summation and retardation tests because both unique inhibitory and common excitatory elements were simultaneously present.

In Experiment 2, an experimental design was employed that, according to the analysis indicated, would maximize the chance of detecting contextual inhibition. Stimuli that were unique to context Y were isolated after training and subsequently used in tests for conditioned inhibition. Contexts X and Y that were used in Experiment 2 differed from one another only in terms of the presence of a different continuously present auditory stimulus. After AX+/BX− differential reinforcement training, in which A and B represent these *contextual* auditory stimuli (they were present throughout the appropriate session), and X refers to the common elements, summation and retardation tests for the associative status of B were performed in contexts that were not used during discrimination training.

An additional experimental manipulation concerned the use of a partly extinguished versus a non-extinguished target in the summation test. Several experiments indicate that especially (partly) extinguished CS are affected by the excitatory strength of the context (e.g., Bouton, 1984; Bouton and King, 1986; Bouton and Peck, 1989). These experiments, as our own Experiment 1A, demonstrate that the excitatory associative strength of contextual stimuli does not enhance responding to a CS if the CS is not under the influence of extinction. Following the same line of reasoning, it was assumed that, perhaps, context Y's potential inhibitory strength can best be detected if the summation test is performed using a partly extinguished target.

3.1. Materials and methods

3.1.1. Subjects and apparatus

The subjects were 16 female Wistar rats with a body weight that ranged from 149 to 206 g. Two of the rats were experimentally naive; the other 14 rats had participated in an experiment in which they had received one unsignalled 1.0-mA, 0.5-s electric footshock, followed by a series of non-reinforced, context-exposure sessions. The box employed in this previous experiment was identical to the shock-reinforced training context XV used in the present experiment (see below). The animals were housed and maintained as described for the previous experiments.

Four different contexts were employed. The box used as context XV in a XV + /XT1 – discrimination training was identical to the first box described in Experiments 1A and 1B, with the exception that a ventilation fan was continuously on and no olfactory cues were deliberately introduced. Context XT1 was identical to context XV, except that the ventilation fan was switched off and an auditory stimulus T1 was continuously presented through the speaker. The contextual auditory stimulus was either a continuous, 75-dB, 7-kHz tone, or an intermittent (1 s on, 1 s off), 75-dB, 1-kHz tone. Two different tones were used because one of the tones, T1, was intended to become a conditioned inhibitor whereas the other tone, T2, was used as a putative neutral test tone (see Section 3.1.2). Thus, the only difference between XV and XT1 was the continuous presence of auditory stimulation produced by either a ventilation fan or a tone.

The box used for the summation test, context Y, was the same box as the second box described in Experiments 1A and 1B, except that illumination was provided by four white fluorescent tubes (light intensity: 48 cd/m^2) and an olfactory cue was provided by smearing approx. 1 g of an anti-mosquito stick (lemon scent) on a piece of paper that was placed underneath the grid floor.

Finally, the retardation test was conducted in the same box as described as the third box in Section 2.1.1 of Experiments 1A and 1B, except for the following. The room was illuminated by three red fluorescent tubes (< 1 cd/m^2) and a distinctive scent was provided by placing a piece of wadding drenched in cleaning alcohol underneath the grids. This box is referred to hereafter as context Z.

A Panasonic low-light camera was placed in front of the different contexts so as to monitor the rats' activity inside. A flashing light used for pacing the scoring of the rats' behaviour was placed in such a manner that it was monitored by the camera, but invisible to the rats. Presentation of the flashing light, tones and US was controlled by a Macintosh/SE computer.

3.1.2. Procedure

An outline of the experimental procedures of Experiment 2 is presented in Table 1.

3.1.2.1. Differential conditioning to contexts. All rats were subjected to a contextual discrimination procedure that lasted 16 experimental days. On each of these days, three different types of session were performed. Each session lasted 4 min. The first type of session was performed in context XV. The animals were individually placed in XV and after a mean of 160.9 s (range: 125–200 s), one

Table 1
Design of Experiment 2

Group	Discrim. training	Training Y	Ext/noExt Y	Summation test	Retardation test
Ext	XV +, XT1 −, HCT2 −	Y +	Y −	YT1, YT2	ZT1 + or ZT2 +
noExt	XV +, XT1 −, HCT2 −	Y +	HC	YT1, YT2	ZT1 + or ZT2 +

X, Y, and Z denote different experimental boxes; V, T1, and T2 designate different continuously present auditory stimuli. + and − refer to, respectively, shock reinforcement and the absence thereof. HC designates the home cage and Ext and noExt represent extinction and no extinction, respectively. Prior to the summation and retardation test, discrimination retraining sessions were conducted that were identical to the sessions in the discrimination training phase (not shown).

0.5-mA, 0.5-s electric shock was presented (XV + sessions). The second session type was performed in context XT1. For one half of the rats, this context consisted of the box with the continuous tone on throughout the session; for the other half, it was the box with the intermittent tone continuously on. The sessions in XT1 merely consisted of placing the rats in the box without experimental manipulations being planned (XT1 − sessions). Finally, the third type of session consisted of transporting each animal in its HC to the experimental room, and placing the HC in front of the box used for the other two sessions, box X, for 4 min. During these 4 min, the lid that covered the environmental chest was left open and the tone that was not used during the sessions in XT1 (for one half of the rats the intermittent tone; for the other half the continuous tone) was on in X. In this manner, the rats were exposed to the alternative tone, hereafter referred to as T2, without being simultaneously exposed to (all) excitatory elements provided by the training box, as was the case during the training sessions in XV and XT1. The purpose of this third type of session, hereafter symbolized as a HCT2 session, was to equate the total time that the rats were exposed to the two different tones. The idea was that, as a result of this training regimen, only the tone that was simultaneously presented with the excitatory elements X and non-reinforced, namely T1, would acquire a conditioned inhibitory property. The other tone, T2, was used as a control stimulus in the summation and retardation tests to be performed later (see below). The order of the daily test sessions was as follows. The first and second sessions of the day were always a XV + or a XT1 − session. The order was semi-random with the restriction that on no more than 2 successive days the first session was of the same type. The HCT2 session was always the third session of the day. The interval between sessions was approx. 1 h. Training sessions were run on weekdays.

3.1.2.2. Conditioning / partial extinction of context Y and discrimination retraining 1. The first part of this phase was intended to establish two kinds of targets to be used in a summation test: a consistently reinforced context and a conditioned then partially extinguished context. Two matched groups of equal size were formed on the basis of discrimination performance and the type of tone used as the putative inhibitory contextual stimulus. Each animal in group Ext ('extinction') and group noExt ('no extinction') received five context Y conditioning sessions. During each of these, the rats were individually placed in Y and a shock US was presented after a mean of 147 s (range: 65–190 s). The shock intensity was 0.5 mA, 0.5 s on conditioning sessions 1 and 2. In order to promote a more rapid increase in freezing, the shock intensity was increased to 0.8 mA in sessions 3–5. Although this increase could result in some generalization decrement in the sense that the US used for inhibitory training was no longer the same as the US used in the context employed for the future summation test (see below), it was assumed that this effect would be rather small and that the benefit of obtaining a high baseline freezing level without conducting a large number of training trials was more important. The interval between sessions was variable and ranged from 2 to 72 h.

Following the last context Y conditioning session, the animals in group Ext received a total of eight context extinction sessions. During each of these, the animals were individually placed in Y for 4 min and no stimuli were presented. The interval between extinction sessions ranged from 1 h to 24 h. The animals in group noExt remained in the HC.

To ensure intact discrimination performance, two additional training sessions were performed in each of the contexts XV, XT1, and HCT2, after the last context extinction session of group Ext. These sessions, which were given to both groups, were identical to the context discrimination training sessions of the first phase of the experiment. The mean placement-shock interval in XV was 157.5 s and the order of sessions was XV + , XT1 − , HCT2, XV + , XT1 − , HCT2.

3.1.2.3. Summation test and discrimination retraining 2. On the first day of the summation test, one half of the animals (counterbalanced for group) was individually placed in context Y for 4 min while T1, the putative inhibitory tone, was continuously on. The other half of the animals received an identical session, except that T2, the putative neutral tone, was on. Twenty-four hours later, the experimental manipulations were identical to those on the previous day, except that the alternative tone was employed. Thus, the first half of the rats mentioned above were now exposed to YT2; the second half to YT1. The purpose of these tests was to examine whether or not freezing to Y would be attenuated by the presence of T1, relative to the session during which T2 was presented.

Continued XV/XT1 discrimination performance was ensured by presenting two standard context discrimination training days. The order of the sessions was XV + , XT1 − , HCT2, XT1 − , XV + , HCT2, and the mean placement-shock interval was 177.5 s.

3.1.2.4. Retardation test. One half of the subjects (counterbalanced for group) received two reinforced tests in context Z with T1 continuously on. Each test consisted of placing the rat in ZT1 and delivering a shock after a mean of 167.5 s. The remaining animals received identical sessions, except that T2 was continuously presented. The interval between sessions was 48 h. The purpose of these sessions was to examine whether conditioning to the ZT1 context would proceed slower than conditioning to the ZT2 context.

As in the previous experiments, the conditioned response observed was freezing. The primary observer, whose data are presented below, was blind to the specific group assignments during the summation and retardation tests. The two 4-min summation tests, as well as the first minute of each of the two retardation tests, were re-scored by a second observer. This latter observer was unaware of the purpose of the experiment. The inter-observer reliability for each of the test sessions was calculated by correlating the total number of freezing responses scored by each observer. The mean correlation coefficient was 0.92 (S.E.M.: 0.03). Statistical analyses using the data of the second observer revealed exactly the same pattern of results as those based on the data reported below.

3.2. Results

3.2.1. Differential conditioning to contexts

After 16 days of training, two animals clearly had still not solved the discrimination. Pooled over all sessions, they even showed less freezing in context XV than in context XT1. Because we were

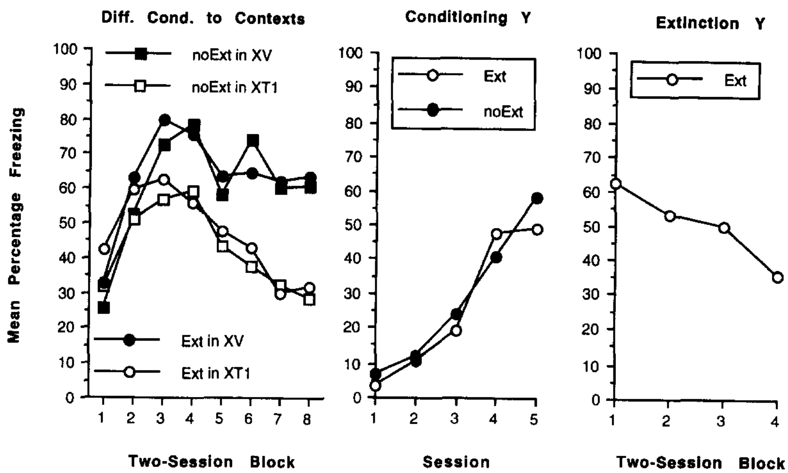

Fig. 2. Left: groups' mean percentages of observations scored as freezing during the first minute of each session of differential conditioning to context XV (reinforced context) and context XT1 (non-reinforced context) in Experiment 2. Group Ext would later receive a series of shocks in a third context, followed by extinction sessions. Group noExt would later receive only the shock phase in the third context. Middle: mean percentages of observations scored as freezing during the first minute of the context Y conditioning sessions for Group Ext and group noExt in Experiment 2. Right: mean percentage of observations scored as freezing during the first minute of each of the two-session context Y extinction blocks for group Ext in Experiment 2.

specifically interested in the associative status of T1 in rats that had solved the discrimination, we decided to exclude these two animals from further analyses. Hence, the data shown below are based on $n = 7$ for each of group Ext and group noExt.

The left side of Fig. 2 shows the results of the contextual discrimination training phase. Although in this phase of the experiment the group membership, Group Ext versus Group noExt, was not yet relevant, the data shown are separated on the basis of this factor to illustrate the degree of discrimination learning in each group.

Fig. 2 shows that from block 3 on, the rats in each group consistently froze more during reinforced XV sessions than during non-shock XT1 sessions. The factor Physical Identity of T1 (continuous or intermittent tone) did not have a significant effect in any of the statistical analyses reported below, and neither did it reliably interact with any other main factors included in the analyses. Therefore, this factor was excluded. A Group × Type of Session (XV + or XT1 −) × Block repeated measures ANOVA performed on the training data revealed a significant main effect of Type of Session ($F(1, 12) = 36.33$) and of Block ($F(7, 84) = 10.15$). The interaction between Type of Session and Block was also significant ($F(7, 84) = 9.37$). On block 1, rats froze more during XT1 − than during XV + sessions, whereas from block 3 on, the animals froze more during XV + than during XT1 − sessions ($Fs(1, 74) > 8.85$). These results indicate that the rats had solved the contextual discrimination.

3.2.1.1. Conditioning / extinction context Y and discrimination retraining 1. The middle and right panels of Fig. 2 show the data from the context Y conditioning phase (both groups) and the context Y extinction phase (group Ext). A Group × Session ANOVA performed on the conditioning data only revealed a significant effect of session ($F(4, 48) = 19.23$), reflecting successful conditioning to Y in

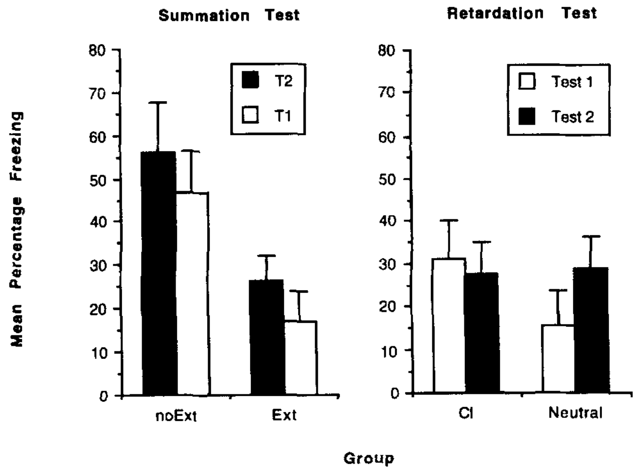

Fig. 3. Left: the results of the summation test in Experiment 2. Groups' mean percentages of observations scored as freezing during the total session in Y with the putative inhibitory tone T1, and the putative neutral tone T2 continuously on. Groups differed in the conditioning history of the target context Y. In group noExt it had consistently be reinforced by shock; in group Ext it had first been reinforced and then non-reinforced. Right: the results of the retardation test in Experiment 2. Mean freezing percentages observed during the first minute of the retardation test sessions for the animals conditioned with T1 (group CI) and T2 (group Neutral) continuously present.

both groups. Partial extinction of conditioned responding to context Y in group Ext was statistically confirmed by a significant effect of Block ($F(3, 18) = 3.96$).

On the first discrimination retraining day, discrimination performance proved to be disrupted as a result of the foregoing context conditioning/extinction sessions. Group noExt froze on 41.7% and 28.6% of the observations made during the first minute of, respectively, the first reinforced (XV +) and first non-reinforced (XT −) retraining session. For group Ext, the corresponding percentages were 38.1 and 35.7. A Group × Type of Session ANOVA on these data revealed no significant effects ($F < 1$). However, the disruption proved to be only temporary. On the second retraining day, group noExt froze on 72.6% and 46.4% of the first-minute observations of the XV + and XT − session, respectively. For group Ext, the corresponding percentages were 69.1 and 45.2. A Group × Type of Session ANOVA only revealed a highly significant effect of Type of Session ($F(1, 12) = 19.46$), indicating that discrimination performance was restored in both groups.

3.2.1.2. Summation test. The left part of Fig. 3 shows the results of the summation test, pooled over the factor Test Order (first YT1 or YT2 session).

As can be seen, group Ext froze less than group noExt on both types of session and, more importantly, both groups showed less freezing during the session with the putative inhibitory tone, T1, continuously on than during the session with the supposedly neutral tone, T2, continuously present. The factor Test Order was not included in the analysis described below because it had no significant effect and did not reliably interact with any other main factors. A Group × Type of Session (YT1 or YT2) ANOVA confirmed the statistical reliability of the between- and within-group differences. The analysis revealed a significant effect of Group ($F(1, 12) = 6.23$), in addition to a significant effect of Type of Session ($F(1, 12) = 5.37$). The interaction between main effects was not significant ($F = 0$).

These results indicate that the extinction manipulation in group Ext had been successful and that both groups showed less freezing during T1 than during T2, irrespective of the specific manipulation regarding context Y.

3.2.1.3. Discrimination retraining 2. The original XV + /XT1 − discrimination performance was still intact in both groups after the summation test sessions. Group noExt froze on 58.3% and 44.1% of the observations during the first minute of the first and second retraining session in XV, respectively. For the non-reinforced sessions in XT1 the corresponding freezing percentages in this group were 20.2 and 14.3. In group Ext, the freezing percentages on XV sessions were 64.3 and 47.6, and during XT1 sessions, 36.9 and 29.8. A Group × Retraining Session × Type of Session ANOVA only revealed a significant effect of Retraining Session ($F(1, 12) = 6.50$), reflecting less overall freezing during the second session than during the first, and a significant effect of Type of Session ($F(1, 12) = 17.56$).

3.2.1.4. Retardation test. The right panel of Fig. 3 shows that for the animals that were conditioned with T2 (Neutral), the putative neutral tone, there was a slight increase in freezing from session 1 to session 2. For the other animals, those that were conditioned with the putative inhibitory T1 (CI), the conditioning experience on test 1 did not result in increased freezing during test 2. A Group (conditioned with T1 or T2 present) × Test ANOVA using the retention test data only revealed a significant interaction between Group and Test ($F(1, 12) = 6.61$). Subsequent simple effects analyses revealed that there was a significant difference between Test 1 and Test 2 freezing in Group Neutral ($F(1, 12) = 8.15$), but not in Group CI ($F < 1$). There was no difference between groups on either test ($Fs(1, 14) < 1.79$).

3.3. Discussion

Discrimination learning proved to be more difficult to establish in Experiment 2 than was the case in either Experiment 1A or 1B. This might be due to the common excitatory elements X in the present contexts XV and XT1 being more salient than was the case for the discrimination training contexts employed in the first two experiments. In Experiment 2, the same box was used as XV and XT1, whereas in the other two experiments, X and Y consisted of different boxes. Given the apparent difference in the ease of discrimination learning, it must then be assumed that the auditory stimulus in the non-reinforced context, on the basis of which the rats could discriminate between the reinforced and non-reinforced contexts in Experiment 2, could not counteract the supposedly salient common elements to the same extent as the unique contextual elements of the non-reinforced context in Experiments 1A and 1B could counteract the probably weaker excitatory common elements present in those experiments. However, this does not imply that the net inhibitory strength of the unique elements of the non-reinforced context was lower in Experiment 2 than in either Experiment 1A or 1B.

In the summation test, irrespective of the differential extinction manipulation concerning the target context Y, the presence of the intended inhibitory tone, T1, suppressed the level of freezing in Y, relative to the level observed in that context in the presence of the putative neutral tone, T2.

One might argue that T1 was functioning as a negative occasion setter instead of as a conditioned inhibitor (see Section 1). However, this is unlikely given the fact that T1 was simultaneously compounded with other contextual cues. This temporal arrangement is not conducive to occasion setting (Holland, 1985, 1986). Furthermore, the fact that T1 equally suppressed freezing in group Ext and group noExt also has implications for the viability of a negative occasion-setting account. Previous research indicates that an excitatory context can sometimes enhance conditioned responding to a target stimulus if a (partially) extinguished stimulus is used as the target (e.g., Bouton, 1993; Rescorla, 1985). One account of such a result is that, as a consequence of the extinction manipulation, the target has become an ambiguous stimulus in that two kinds of associations co-exist: a target-US and a target-no-US association. It might be that such a target is especially susceptible to modulation by a positive occasion setter because the latter has been trained in a procedure in which the original target also had a mixed history of reinforcement: it was reinforced in the presence of the intended occasion setter and non-reinforced in its absence. In this sense, the original target and the novel transfer target are similar. Although currently there is little empirical support that negative occasion setters also transfer their inhibitory power to novel, partly extinguished targets (but see Swartzentruber and Rescorla, 1994), one would expect that, in case transfer *does* occur, it would probably be detected more easily with partly extinguished targets than with consistently reinforced targets. Accordingly, the absence of an effect of the extinction manipulation in the present experiment, which results in the target context acquiring an ambiguous meaning, is not expected if T1 was a negative occasion setter.

The inference that T1 was functioning as a conditioned inhibitor was strengthened by the results of the retardation test, a test that does not require reference to the rather complex literature on transfer and summation of contextual associative strengths. Although the effect was rather small, a significant difference in the effect of the presence of T1 versus T2 was found. This positive finding suggests that the null result of Experiment 1B was caused by the combined use of Y's excitatory and inhibitory elements in that experiment.

4. General discussion

Experiments 1A and 1B showed that a X + /Y − differential context-conditioning procedure did not result in context Y as a whole acquiring an inhibitory property as assessed by a summation and retardation test. In Experiment 2, an identical training procedure was used. However, subsequent tests for conditioned inhibition were performed using a contextual stimulus that was unique to Y. This unique contextual stimulus passed a summation test using both a conditioned context and a conditioned then partially extinguished context as a target stimulus. Furthermore, it did also pass a retardation test.

These results suggest that a contextual stimulus can become a conditioned inhibitor through differential context conditioning, but that detection of this inhibition requires isolation of the elements that are unique to the non-reinforced context. If the whole array of stimuli that constitute the non-reinforced context is examined, the inhibition acquired by the unique elements is masked by the excitatory elements that this context has in common with the reinforced context.

There was a difference between Experiments 1A and 1B, on the one hand, and Experiment 2, on the other, in both the nature of the intended contextual inhibitor (non-auditory vs. auditory contextual stimulus) and the nature of the target stimulus employed during the summation test (discrete stimulus vs. contextual stimulus). This means that the different findings do not necessarily demand an explanation as proposed above. However, a conceptualization of our experiments in terms of the acquisition of both simple excitatory and inhibitory contextual associations as described by the Rescorla–Wagner model (Rescorla and Wagner, 1972) for classical conditioning, fits nicely with the empirical results. Accordingly, the present training procedure may be schematically outlined as a AX − , AX + , BX − , BX − procedure, in which A and B represent unique contextual stimuli (continuously present auditory stimuli), and X signifies the common contextual elements. The AX compound was both reinforced (AX +) and non-reinforced at times when the shock was not delivered (AX −) and for each AX + trial, there was also a corresponding BX − 'trial', and for each hypothetical AX − trial there was also an (additional) BX − trial. Simulations by means of the Rescorla–Wagner model reveal that, irrespective of the precise values for the different parameters in this model, X possesses excitatory associative strength at all stages of discrimination training. This excitation will be detrimental to detecting inhibitory strength acquired by B if tests for inhibition are performed in the presence of X.

Another possible evaluation of contextual inhibition in the present X + /Y − procedure is in terms of stimulus configuration. A recent model by Pearce (1987) may be examined as one example of a configural account. Accordingly, contexts X and Y may each be regarded as a collection of stimuli that are perceived as one unique, or configural stimulus, and not as the sum of separate elements, that is, a stimulus compound. However, these two configural stimuli must be assumed to share common elements that are responsible for generalization from one context to the other of excitatory and inhibitory associative strengths that are acquired in the process of discrimination learning. Thus, context X may be conceptualized as consisting of configural stimulus AX and context Y of configural stimulus BX. The common 'X' element in each stimulus configuration provides the basis for generalization between contexts. Application of the computational rules offered by Pearce (1987) to an AX − /AX + /BX − /BX − procedure reveals that configural stimulus BX will have a net associative strength that equals 0. However, the separate B element has a net negative associative strength. This is because, for this element, there is more generalized inhibition, namely from BX and XA, than there is generalized excitation from XA. Hence, inhibition will only be detected with regard to the element that makes context Y different from context X. Collectively, for predictions regarding the present procedure, it makes no difference whether the contexts are treated as the compounding of separate elements or as configural stimuli.

It must be noted that from an analysis in terms of the Rescorla–Wagner model, it does not necessarily follow that in a standard A + /B − differential inhibition procedure with discrete stimuli, conditioned inhibition is also constantly masked by concurrently present excitatory stimuli. Suppose that in a differential inhibition experiment one discrete stimulus, stimulus A, is consistently reinforced in context X, and another discrete stimulus, stimulus B, is consistently non-reinforced, also in X. In such a procedure, the element that is common to both reinforced and non-reinforced sessions, context X, is also repeatedly 'presented' in the absence of A and B and not reinforced (X −). Simulations of the changes in the associative strength of A, B, and X in an X − /AX + /X − /BX − procedure by means of the Rescorla–Wagner model, yield that, at asymptote, stimulus A has acquired an asymptotic excitatory strength, whereas both B and X have an associative strength that equals 0. However, pre-asymptotically, and especially under the condition in which the salience of X is larger than that of both stimulus A and B, a situation occurs in the process of discrimination training in which B still has inhibitory strength, whereas the context has already become neutral. This means that, as long as training is not continued long after conditioned responding to the BX compound has ceased (see Hammond, 1968), B can demonstrate an inhibitory potential even if it is tested in the original training context X.

In any case, the present Experiment 2 provides a first indication of the existence of contextual inhibition after differential reinforcement of two contexts. Further research is necessary to assess the generality of the present findings using other types of contextual stimuli as putative inhibitors, and different types of transfer targets in summation tests.

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